The latter part of the paper deals with the extension of Paschen's law to spark lengths much shorter than those actually used in the experiments, and evidence is adduced in support of the conclusion that the law is applicable for discharges in a uniform field in any gas, as long as the spark length is greater than the diameter of the sphere of molecular action.

"On the Optical Activity of Hæmoglobin and Globin." By Arthur Gamgee, M.D., F.R.S., Emeritus Professor of Physiology in the Owens College, Victoria University, and A. Croft Hill, M.A., M.B., late George Henry Lewes Student in Physiology. Received January 31,—Read February 12, 1903.

Introductory Observations.

All observations hitherto published concerning the optical activity of the albuminous substances have led to the conclusion that the bodies thus designated, whether derived from the vegetable or the animal kingdoms, without a single exception, deviate the plane of polarisation to the left, no case having hitherto been known either of a dextrogyrous, a racemic, or an otherwise inactive albuminous substance.*

There is one group of albuminous substances which, notwithstanding the fact that it includes bodies of paramount physiological and chemical interest, has hitherto been completely neglected, in so far as the investigation of the optical activity of its members is concerned. The group to which we refer is that which has been designated by German writers the group of the "Proteïde." This group comprises those complex albuminous substances which can, with greater or less ease, be split up into, or which yield as products of decomposition, on the one hand, albuminous bodies, and on the other, such bodies as colouring matters, or nucleins and nucleinic acids and the purin-bases which result from the decomposition of the latter. The best

* Whilst this paper was being printed, it has come to our knowledge that the late Professor Alexander Schmidt, of Dorpat, described under the name of Cyto-globin, what was certainly a mixture of impure nucleoproteids which he separated from the soluble constituents of many animal cells. He definitely recognised the dextrorotatory properties of this product. For information on A. Schmidt's work, the reader is referred to the "Supplementary Bibliographical Note" at the end of the paper by Gamgee and W. Jones "On the Nucleoproteids of the Pancreas' Thymus, and Suprarenal Gland, with especial reference to their Optical Activity." *Infra*, p. 385.—*March* 5.

characterised and the most striking members of this group are: Firstly, the hæmoglobins and their compounds. Secondly, the nucleoproteids and the nucleins.

In hæmoglobin, we have the example of a complex proteid, which differs from all other members of the albuminous group of bodies by its colour, by its marvellous power of forming easily dissociable compounds with oxygen and certain other gases, by the facility with which it admits of being crystallised and recrystallised and obtained free from all foreign mineral matters, by the startling manner in which its solutions fail to furnish any one of the reactions characteristic of albuminous substances in solution, *so long as the reagent has not effected a fundamental decomposition which has liberated the albuminous and coloured residues.* The researches of one of us have moreover lately shown that whilst hæmoglobin is a diamagnetic body, the iron-containing products of its decomposition by acids are not merely paramagnetic, but probably the most powerfully "ferromagnetic" organic bodies known to science.*

So complete a divergence thus exists in physical and chemical properties between hæmoglobin and the substances which are the immediate products of its decomposition, and which are doubtless linked together in it, that it appeared in the highest degree interesting to ascertain whether or not, in respect to optical activity, hæmoglobin would behave as an albuminous body proper and prove to be "lævogyrous." Having, if possible, determined this point, the subsequent step in the research would naturally be to determine the optical activity of the albuminous and coloured products of the decomposition of the hæmoglobin molecule.

1.—*Determination of the Optical Activity of Hæmoglobin.*

So far as the authors have been able to ascertain, the optical activity of solutions of coloured organic bodies has not yet formed the object of serious investigation. Landolt,† in the last edition of his authoritative work, which contains all the reliable results relating to the optical activity of organic bodies up to the date of its publication, mentions only one colouring matter as having been investigated, viz., the vegetable colouring matter hæmatoxylin, of which the alcoholic solution is said to be dextrogyrous. Nor is this neglect of the study

* A. Gamgee, "On the Behaviour of Oxy-hæmoglobin, Carbonic-oxide-hæmoglobin, Methæmoglobin, and certain of their Derivatives in the Magnetic Field, with a Preliminary Note on the Electrolysis of the Hæmoglobin Compounds," 'Roy. Soc. Proc.,' vol. 68, p. 503.

A. Gamgee, The Croonian Lecture for 1902, "On certain Chemical and Physical Properties of Hæmoglobin," 'Roy. Soc. Proc.,' vol. 70, p. 79.

† Dr. H. Landolt, 'Das Optische Drehungsvermögen Organischer Substanzen, &c., Zweite gänzlich umgearbeitete Auflage,' Vieweg u. Sohn, 1898.

of the optical activity of coloured solutions surprising when we consider the much greater difficulties which encounter the observer, in comparison to those attending the examination of colourless solutions.

The Method Employed in the Present Research.

When a powerful beam of white light is passed through a stratum 1 cm. thick of a solution of Oxy- or CO- hæmoglobin containing 0·9 per cent., the only region of the spectrum which is unabsorbed is that which extends from B to a little distance on the red side of D. It was therefore clear that the only light which could be employed in the work before us was monochromatic red light, and that in the place of one of the polarimeters commonly employed in our laboratories and whose adjustments only permit of their being employed with light of a definite wave-length (the half-shadow polarimeters of the Laurent type being adjusted for use with monochromatic sodium light), an instrument should be employed, the arrangements of which permit of observations with light of any desired wave-length.

In our first observations, we attempted to employ the lithium flame as our source of light, but we were unable to secure by this means either a sufficiently powerful or steady illumination. We subsequently employed, however, as a source of practically monochromatic red light, the light of an arc lamp which had traversed Landolt's filter for red rays.

This light filter consists of a double cell, each compartment of which has a depth of 20 mm. One compartment is filled with a solution of hexamethylpararosanilin, a substance sold commercially under the name of "Crystal Violet 5 BO." 0·05 gramme of this compound is dissolved in a small quantity of alcohol and the solution is then diluted with water to the volume of one litre. When light is made to traverse a stratum 20 mm. thick of this solution, its spectrum consists of a narrow red band and a broad blue-violet part. If, however, the second compartment of the double trough contains a solution made by dissolving 10 grammes of potassium chromate in 100 c.c. of distilled water, the blue-violet is entirely absorbed and the spectrum of the light which has traversed the two compartments of the light-filter consists of a narrow strip, extending from λ 718 to λ 639$\mu\mu$, where it ends abruptly. The mean wave-length ("optischer Schwerpunkt") corresponds to 665$\mu\mu$, the wave length of C being 656·3$\mu\mu$.*

By means of the above method we secured a beam of red light having a mean wave-length approximately the same as that of C and of sufficient intensity to allow us to make observations on solutions of hæmoglobin containing $\pm$ 1 gramme in 100 c.c. of distilled water, the tubes employed in different sets of observations being 100 mm. and 200 mm. in length.

* Landolt, *op. cit.*, pp. 387—390.

The polarimeter employed in these observations was a magnificent Lippich's "Halbschatten-Polarimeter," with tripartite field of vision, made by Schmidt and Haensch, of Berlin, and belonging to the Davy-Faraday Laboratory of the Royal Institution of Great Britain.

*The Hæmoglobin Employed.**

The solutions of hæmoglobin employed for the determinations of which the results will be given below, were prepared with oxy-hæmoglobin of remarkable purity which had been obtained from the blood of the horse by following the best of the methods (the third method) described by Zinoffsky.†

Two preparations of hæmoglobin made on a large scale and at the interval of some months one of the other were employed. The preparation employed to make the solution of Oxy-hæmoglobin had been crystallised three times, the product of each successive crystallisation having been many times washed with ice-cold distilled water of which the purity was controlled by determining its electrical resistance. This solution contained 2·446 grammes of hæmoglobin in 100 c.c. For polarimetric observations this solution was diluted with an equal volume of distilled water, the dilute solution examined containing, therefore, 1·223 gramme of oxy-hæmoglobin in 100 c.c.

The preparation employed to make the solution of CO-hæmoglobin had been crystallised four times. The crystals of each successive crystallisation had been subjected to washing with pure distilled water as stated above, the solution of the washed crystals of the fourth crystallisation having been saturated with CO. This solution contained 1·84 grammes of dry CO-hæmoglobin. For polarimetric measurement this solution was diluted with an equal volume of distilled water; the dilute solution contained, therefore, 0·92 gramme of CO-hæmoglobin in 100 c.c.

Hæmoglobin, whether in Combination with Oxygen or Carbonic Oxide, is Dextrorotatory.

A. *Oxy-Hæmoglobin.*

The diluted solution of Oxy-hæmoglobin, previously referred to, was employed. This solution, containing 1·223 gramme of hæmoglobin in 100 c.c., was thoroughly saturated with oxygen before being subjected to polarimetric observation.

* The part of Section 1 of this paper which follows has been recast, and the observations described under A and C added, since this paper was submitted to the Royal Society.—*March* 5.

† Zinoffsky, O., "Ueber die Grösse des Hæmoglobinmolecüls," 'Zeitschrift f. physiol. Chemie,' vol. 16 (1886), p. 23.

The tube employed in all the sets of observations measured 1 decimetre.

Three sets of observations were made.

	Observed angle.	Specific rotation $(\alpha)_C$.
1. Mean of first set of observations ...	$+0^\circ\cdot12$	$+9^\circ\cdot8$
2. „ second set of observations	$+0^\circ\cdot125$	$+10^\circ\cdot2$
3. „ third „ „	$+0^\circ\cdot1225$	$+10^\circ\cdot0$

From the above observations we conclude that the specific rotation of Oxy-hæmoglobin for light of the mean wave length of C, $(\alpha)_C = +10^\circ\cdot0 \pm 0^\circ\cdot2$.

B. *CO-Hæmoglobin.*

The diluted solution of CO-hæmoglobin, previously referred to, was employed. This solution contained 0·92 gramme of CO-hæmoglobin in 100 c.c.

Two sets of observations were made with this solution; in the first set a tube 1 decimetre long, and in the second a tube 2 decimetres long being employed.

	Length of tube.	Observed angle.	Specific rotation $(\alpha)_C$.
1. Mean of first set of observations	1 decim.	$+0^\circ\cdot098$	$+10^\circ\cdot65$
2. „ second „ „	2 „	$+0^\circ\cdot203$	$+11^\circ\cdot03$

Taking the mean of the two series of observations we obtain as the specific rotation of a solution containing 0·92 gramme of CO-hæmoglobin in 100 c.c.

$$(\alpha)_C = +10^\circ\cdot8.$$

When the feeble rotatory power of hæmoglobin is considered, the agreement between the results of the investigation of the rotatory power of Oxy- and CO-hæmoglobin must be considered satisfactory and as pointing to the conclusion that the molecule of oxygen or carbonic oxide in combination with hæmoglobin does not influence its specific rotation. The correctness of this conclusion has been established by direct experiment.

C. *The same Solution of Hæmoglobin saturated with O and with CO Compared.*

With the object of determining by direct experiment whether the dissociable combinations formed by O and by CO with hæmoglobin had any influence on its specific rotation, the solution of Oxy-hæmoglobin which served for three sets of observations recorded under A, and which contained 1·223 gramme in 100 c.c. of water, was again experimented with. One portion of this solution was saturated with

oxygen; another portion was agitated with pure CO so as completely to expel the oxygen from its combination with hæmoglobin and replace it by carbonic oxide. In this way were obtained two solutions of hæmoglobin identical in so far as the quantity of colouring matter which they contained, but differing in the fact that in the one case the hæmoglobin was in combination with oxygen and in the other with CO. The solutions were examined in tubes of the same length under the same conditions of illumination. The result was to show that the rotations were identical in the two cases, having the mean value represented by the specific rotation $(\alpha)_C = +10^{\circ}{\cdot}0$.

It is to be remarked that the observations recorded under A and C were carried out subsequent to those on CO-hæmoglobin recorded under B. In the case, particularly, of observations A, the intensity and steadiness of the monochromatic red light employed was, in consequence of the experience previously acquired, more satisfactory than in observations B. We are therefore inclined to consider the numbers expressing the specific rotation of hæmoglobin which we have obtained as the result of observations A to be most worthy of confidence. We do not pretend that these numbers may not need slight modification as the result of future work, though we believe that they are a very close approximation to the truth.

2.—*Determination of the Optical Activity of Globin.*

Preyer gave the name of Globin to the albuminous product of the spontaneous decomposition of hæmoglobin, without, however, being able to furnish any precise account of its properties, its chemical composition, or its relationship to other albuminous bodies. A comparatively recent investigation which we owe to Fr. N. Schulz,* and the results of which have been substantially confirmed by Ivar Bang,† has placed us in possession of valuable and suggestive facts concerning the main albuminous product resulting from the decomposition of hæmoglobin. He has shown that when a solution of hæmoglobin is decomposed by the addition of small quantities of hydrochloric acid, it yields, as main products, 4·2 per cent. of hæmatin and 86·5 per cent. of a characteristic albuminous substance for which he retains the name of globin. He has shown that this substance belongs to the class of "the Histons," so that it would have been preferable, in our opinion, if Schulz had applied to his new body such a name as "Hæmato-Histon," which would have indicated both its origin and its affinities.

Schulz's method of preparing globin, as described by him, is essen-

* Schulz, Dr. Fr. N., "Die Eiweisskörper des Hæmoglobins," 'Zeitschr. f. physiol. Chemie, vol. 24 (1898), p. 449.

† Bang, Ivar, "Studien über Histon," 'Zeitschr. f. physiol. Chem.,' 1899, p. 463.

tially as follows: to a solution of crystallised hæmoglobin, either prepared by Hoppe-Seyler's method or by the ammonium sulphate method, dilute hydrochloric acid is added in extremely small quantities, until a flocculent brown precipitate falls which is immediately dissolved by the slightest excess of acid. The solution then no longer exhibits the beautiful red colour of hæmoglobin, but has assumed a brown colour. Not only, remarks Schulz, has its colour changed, but a complete separation has occurred between the albuminous and coloured constituents of hæmoglobin. If to the solution, which has now a faint acid reaction, about one-fifth of its volume of 80 per cent. alcohol be added and the mixture be shaken with ether, the whole [*sic*] of the colouring matter is taken up by the ether, whilst the subjacent aqueous-alcoholic, perfectly clear solution contains the decolourised albuminous matters. Schulz gives particular directions as to the precautions which must be taken in order that the separation of the ethereal solution of the colouring matter should be complete, stating that a certain relation must exist between the proportions of water, alcohol, and ether, which must be experimentally determined in each case. By the above process there is obtained a more or less brownish-yellow solution, containing both alcohol and water and having a faintly acid reaction. On neutralising this solution with ammonia, a faintly yellow, coarsely flocculent precipitate falls. The latter is rapidly separated by filtration and then washed with water. When the excess of ammonia has been removed, the precipitate commences to dissolve in the wash water. At this stage, the precipitate is dissolved in water with the aid of a few drops of dilute acetic acid. Solution occurs rapidly and completely. The excess of acid is now removed by dialysis continued for some days, the dialyser being surrounded by distilled water. There is thus obtained a clear, odourless and tasteless solution of globin the reaction of which is perfectly neutral.

It is not our object to examine in this place the reactions presented by solutions of globin, and which have led Schulz to place it among the "Histons."

Before describing briefly the methods we employed to prepare the solutions of globin which we investigated optically, we desire to make certain observations on certain points in Schulz's statement. In discussing the quantity of dilute hydrochloric acid needed to effect the decomposition of hæmoglobin, he merely remarks that it is extraordinarily small ("Die zu der Spaltung erforderliche Menge von Säure ist ausserordentlich gering, &c."). We have determined the quantity of decinormal hydrochloric acid required to effect the decomposition of a solution of CO-hæmoglobin of known composition. As a result of very careful experiments with a solution containing 1·84 grammes dissolved in 200 c.c. of water, there were required

20 c.c. of decinormal hydrochloric acid to effect the complete separation of globin from the colouring matter.

We found that agitation with ether, unless repeated several times, fails to remove all the colouring matter which is capable of removal in this way. Further, we found that even when, as a result of agitation with ether, the aqueous-alcoholic solution of globin is of the faintest straw colour, on being neutralised with ammonia the precipitated globin, which is at first colourless, assumes a somewhat reddish tinge, and when subsequently dissolved in water faintly acidulated with acetic acid the solution is much more deeply coloured than the original aqueous-alcoholic solution.

The following is the precise method which we followed in preparing the solutions employed in our polarimetric determinations :—

100 c.c. of a solution of four times crystallised hæmoglobin, containing 1·84 grammes of the substance, was diluted with 100 c.c. of distilled water and treated with 20 c.c. of decinormal hydrochloric acid. 44 c.c. of absolute alcohol were then added to the liquid, which was placed in a stoppered separating funnel and thoroughly agitated with its own volume of ether. The aqueous-alcoholic liquid having been separated from the supernatant ethereal solution of colouring matter was twice more agitated with fresh quantities of ether. By proceeding as we have described, the separation of the solution of globin occurred completely after the first agitation with ether, and the solution after the third agitation only possessed a faint straw colouration. In certain cases, the globin was separated according to the method of Schulz by precipitation with ammonia, the flocculent precipitate being subsequently dissolved in very weak acetic acid. In this manner was prepared the solution of globin which served for the first set of determinations recorded below. As it was impossible to obtain in this way solutions sufficiently colourless to allow of their rotation to be determined satisfactorily for light of the wave-length of D, this was done as in the case of hæmoglobin for light of the mean wave-length of C. In the second set of observations, the rotation of the aqueous-alcoholic solution resulting from the decomposition of hæmoglobin, after thorough agitation with ether, was determined.

Globin a Lævorotatory Substance.

Preliminary observations having shown that solutions of globin are optically active and lævogyrous, the following sets of observations were made with the object of determining the specific rotation of solutions of the substance.

1. A solution of globin in distilled water, but containing a little acetic acid, was examined with the arrangement for red light, as was used in the case of hæmoglobin. The solution contained 2·4 grammes

of globin in 100 c.c. It exhibited in the most characteristic manner the reactions of globin.

The tube employed measured 1 decimetre. The angle of rotation (mean of many determinations) was $-1^{\circ}\cdot30$.

From the above data, it follows that in the case of this feebly acid solution of globin, containing 2·4 per cent., the specific rotation $[\alpha]_C = -54^{\circ}\cdot2$.

2. The faintly straw-coloured solution obtained by the decomposition of hæmoglobin by means of dilute hydrochloric acid, the addition of alcohol and repeated agitation with ether, was placed in a shallow capsule in a current of air for some hours and afterwards on the water-bath at the temperature of 40° C. In this way all the ether and some of the alcohol were expelled. The perfectly clear straw-coloured solution, which had a density of 987·4 at 16° C., contained 0·98 gramme of solid matter in 100 c.c.

Monochromatic sodium light was employed in the polarimetric observations. The tube employed measured 1 decimetre. The angle of rotation (mean of many determinations) was $-0^{\circ}\cdot64$.

From the above data, it follows that in the case of this feebly acid, aqueous-alcoholic solution of globin, containing 0·98 per cent. of solids the specific rotation, $[\alpha]_D = -65^{\circ}\cdot5$. It may be pointed out that the greater part of the discrepancy between the results of the polarimetric measurements of the solution of separated globin and of the solution now under discussion is to be explained by the difference in the wave-length of the light, of which the rotation of the plane of polarisation was determined in the two cases.

General Conclusions.

The following are the conclusions to which we have been led by the experiments described in this paper :—

1. Hæmoglobin is a dextrogyrous albuminous body.

2. Globin, which is the principal, or as we are inclined to believe, the only albuminous product of the decomposition of hæmoglobin by highly dilute hydrochloric acid under the conditions determined by Schulz and confirmed by our own observations, behaves as a normal albuminous substance, in respect to its influence on the plane of polarisation of light, *i.e.*, it is a lævogyrous body.

Whilst the conclusions above stated are beyond question correct, we wish it to be understood that the numbers expressing the specific rotation of the bodies which we have examined must be looked upon as very close approximations and may need revision in the case of hæmoglobin by determinations carried out with a more perfectly monochromatic and intense light than that which we have employed, and in the case of globin by working with the substance in a purer condi-

tion than is possible in the actual state of our knowledge of this body.

We hope to be able to carry out these further investigations, and to direct our attention to the optical activity of the coloured products of the decomposition of the hæmoglobin molecule, especially hæmochromogen and hæmatin and their coloured derivatives.

In conclusion, we have to express our thanks to the Managers of the Davy-Faraday Laboratory of the Royal Institution for the facilities which they afforded us in carrying on the optical part of our work.

"On the Nucleoproteids of the Pancreas, Thymus, and Suprarenal Gland, with especial Reference to their Optical Activity." By ARTHUR GAMGEE, M.D., F.R.S., Emeritus Professor of Physiology in the Owens College, Victoria University, and WALTER JONES, Ph.D., Associate Professor of Physiological Chemistry in the Johns Hopkins University. Received February 9,—Read February 12, 1903.

PART I.—BIBLIOGRAPHICAL AND CRITICAL.

In a research in which one of us was associated with Dr. A. Croft Hill, it was discovered that Hæmoglobin is a dextrorotatory body, whilst the interesting Histon-like albuminous substance Globin, which is obtained by the splitting up of Hæmoglobin under the influence of highly diluted hydrochloric acid, and of which the characters, no less than the mode of preparation, have only been known since the researches of Fr. N. Schulz, is a normally lævogyrous albuminous body.

These interesting observations naturally suggested the probability that the Nucleoproteids might, like Hæmoglobin, prove to be dextrogyrous, and the research of which the first results are contained in this paper is the outcome of this idea. The hypothesis has been fully confirmed, as will be shown in the sequel, and it has thus been proved that some of the members of a group of albuminous bodies of great importance in the life-history of the organism, are dextrorotatory bodies.

The preparation of nucleoproteids of such purity and especially so free from contaminating colouring matters as to yield solutions sufficiently transparent and colourless for polarimetric work, was a necessary preliminary to our special researches, and has led to the discovery of many facts of interest in relation to the chemistry of the nucleoproteids.